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HLA-DR antigen linkage of anti-β receptor antibodies in idiopathic dilated and ischaemic cardiomyopathy

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Abstract

***Objective*—Immunological mechanisms have been implicated in the pathogenesis of human dilated cardiomyopathy. The presence of autoantibodies against the β_1 adrenoceptor in a substantial proportion of patients with dilated cardiomyopathy has been described and an association between the HLA-DR4 phenotype and anti-β receptor antibodies has been identified. The objective of the present study was to examine whether the presence of such antibodies in ischaemic cardiomyopathy was limited to specific HLA-DR phenotypes.**

***Design*—The HLA-DR dependence of anti-β receptor antibodies detected by a ligand binding inhibition assay in patients with dilated cardiomyopathy (n = 68) was compared with that in patients with ischaemic cardiomyopathy (n = 73).**

***Results*—38% of the patients with dilated cardiomyopathy and 22% of those with ischaemic cardiomyopathy had serum anti-β receptor antibodies. In dilated cardiomyopathy, the presence of anti-β receptor antibodies was linked to the HLA-DR4 phenotype (that is, 50% of patients with this phenotype were antibody positive) whereas, in those with ischaemic cardiomyopathy HLA-DR1 was over-represented (that is, 37% of the patients with the HLA-DR1 phenotype were antibody positive compared with 17% of the HLA-DR1 negative patients). In both disease entities, the HLA-DR3 phenotype was virtually absent in the anti-β receptor antibody group.**

***Conclusions*—These results suggest that the presence of anti-β receptor antibodies is under immune genetic control that may depend on the nature of the underlying disease process.**

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Abnormalities in both cellular and humoral immunity have been repeatedly described in human dilated cardiomyopathy and support the involvement of autoimmune mechanisms in the pathogenesis of this disorder.[1-3] The specific targets of the presumed autoimmune response have not been clearly defined, but are thought to include several cell surface and intracellular constituents. Recently, we,[4] as well as others,[5] have described the presence of antibodies directed against the β_1 adrenergic receptor in a substantial proportion (about 30%) of patients with idiopathic dilated cardiomyopathy. These antibodies inhibit both ligand binding to membrane β receptors[4] and isoproterenol sensitive adenylate cyclase activity.[6] Their presence is linked to the HLA-DR4 phenotype[6,7] suggesting that the increased frequency of this phenotype in dilated cardiomyopathy may reflect the presence of immune genetic factors controlling the propensity to develop autoantibodies.

The frequency of anti-β receptor antibodies in ischaemic heart disease is much lower than that in idiopathic dilated cardiomyopathy.[4] Furthermore, it is not known whether the same immune genetic influences are responsible in both diseases, that is whether the preponderance of HLA-DR4 in antibody positive patients is linked to the propensity to develop autoimmunity or to the pathogenesis of the disease. This issue is examined in the present study by comparing the distribution of HLA-DR antigens in patients with dilated cardiomyopathy and with ischaemic heart disease patients both with and without anti-β receptor antibodies.

Patients and methods

We studied 141 patients (aged 38–82, mean 49 for the dilated cardiomyopathy and 53 for the ischaemic cardiomyopathy group) evaluated at the University of Minnesota. In 68 (64 men and four women) the diagnosis was idiopathic dilated cardiomyopathy and in 73 (67 men and six women) it was ischaemic heart disease. All patients had undergone routine clinical and haemodynamic evaluation, including coronary arteriography and cardiac catheterisation, and were similar in severity of cardiac dysfunction. Phenotyping for HLA-DR was carried out using standard lymphocytotoxicity assays as previously described.[8] Statistical evaluation was as described by Svejgaard *et al.*[9] p Values were corrected for the number of antigens tested and associations were judged significant if $p < 0.05$.

The assay for β receptor autoantibodies is based on their ability to inhibit ligand binding to membrane β adrenoreceptors and has been previously described.[4] Briefly, rat cardiac membranes (0·1 to 0·2 mg protein) were incubated with 100-fold serum dilutions in 50 mM Tris-HCl (pH 7·5), 5 mM MgC_{12}, 0·1 mM phenylmethyl-sulphonylfluoride,

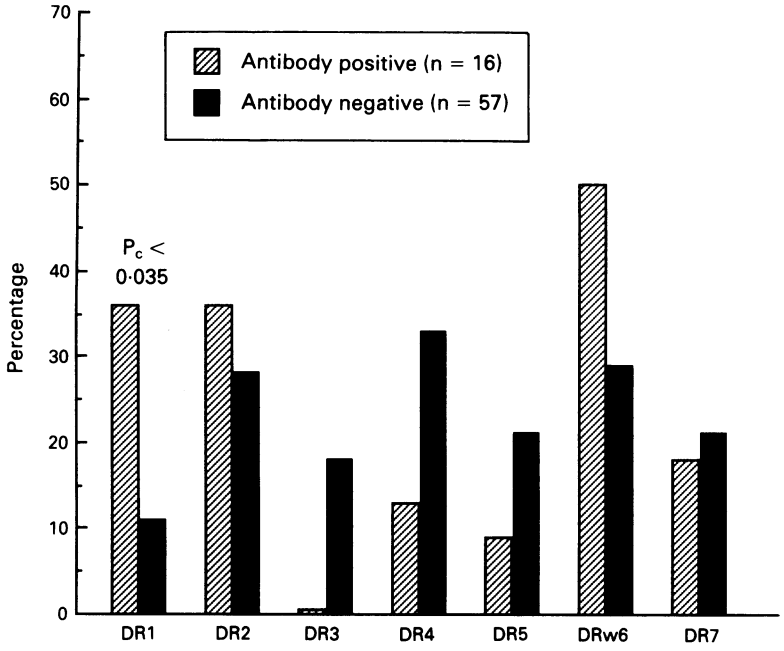

Figure 1 Distribution of HLA-DR antigens in patients with ischaemic cardiomyopathy with or without anti-β receptor antibodies as defined by ligand binding inhibition assay.

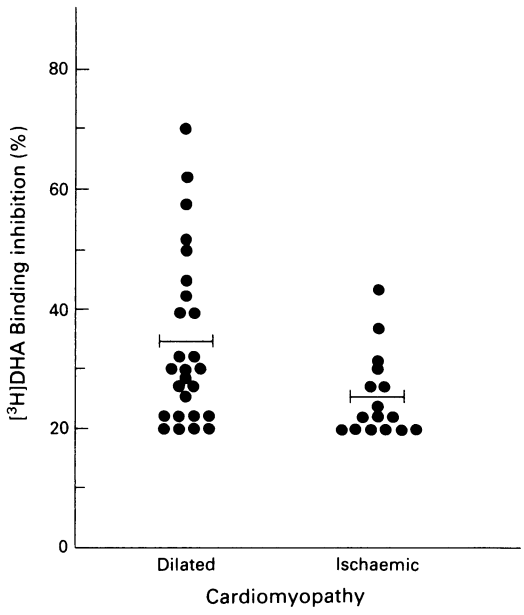

Figure 3 Extent of [3H]dihydroalprenolol (DHA) binding inhibition by 100 fold serum dilutions from patients with dilated or ischaemic cardiomyopathy. The extent of inhibition is related to [3H]DHA binding to control cardiac membranes (incubated in the absence of serum).

5 μg/ml leupeptin, and 7 μg/ml pepstatin at 30°C for 60 min, and then with 6 nM [3H] dihydroalprenolol (New England Nuclear Co, specific activity 105 Ci/mmol) for 15 min. Reactions were terminated by filtration through Whatman GF/C fibres. Non-specific binding was determined in the presence of 1 μM propranolol. Positive results were defined as ⩾20% inhibition of ligand binding in the presence of 100-fold serum dilution.

Results

Anti-β receptor antibodies were present in 38% (26 of 68) of patients with dilated cardiomyopathy compared with 22% (16 of 73) of patients with ischaemic heart disease. Figure 1 shows the distribution of HLA-DR antigens in antibody positive and negative patients with ischaemic cardiomyopathy. There is a significant over-representation of HLA-DR1 phenotype (37% of antibody positive subjects typed as HLA-DR1 compared with 11% of those who were antibody negative (Pc < 0·035)) while DR3 is distinctly less frequent. By comparison, anti-β receptor antibody positive patients with dilated cardiomyopathy were more likely (Pc < 0·02) to belong to the HLA-DR4 phenotype (fig 2). Although two thirds (6/9) of the HLA-DR1 patients were antibody positive, the numbers were too small to reach statistical significance.

In an alternative approach the antibody status of the HLA-DR1 positive and negative patients was considered. In ischaemic cardiomyopathy, 37% (6/16) of the HLA-DR1 positive patients had anti-β receptor antibody compared with 17% (10/57) of the HLA-DR1 negative patients. In dilated cardiomyopathy 50% of the HLA-DR4 patients were antibody positive while only 24% of the HLA-DR4 negative patients were antibody positive. In both disease groups, HLA-DR3 was essentially absent in the antibody positive group. There was a small but significant difference (p < 0·05) in the extent of ligand binding inhibition between ischaemic (24·7 (2·0)%) and dilated cardiomyopathy (33·5 (3·0)% groups (fig 3).

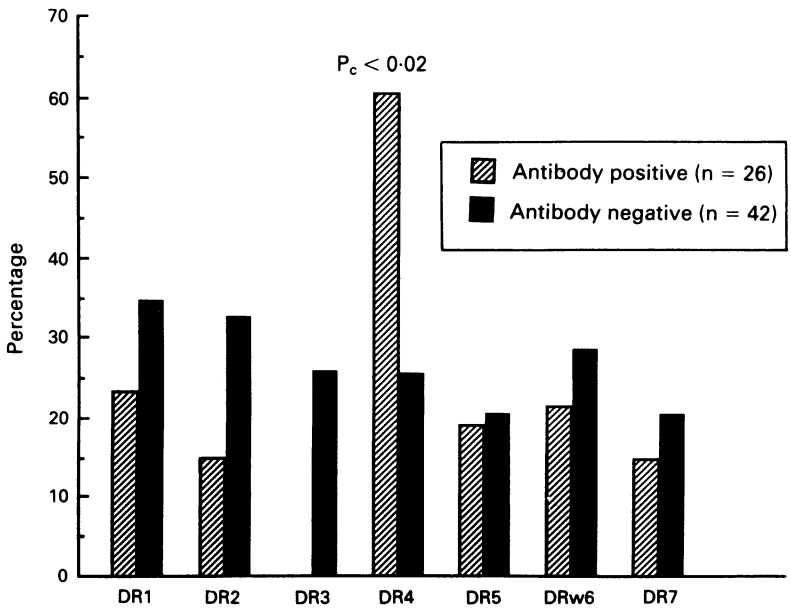

Figure 2 Distribution of HLA-DR antigens in patients with dilated cardiomyopathy with or without anti-β receptor antibodies as defined by ligand binding inhibition assay.

Discussion

We have recently shown that a substantial proportion of patients with idiopathic dilated cardiomyopathy have autoantibodies directed against their β adrenoreceptors as judged by their ability to inhibit ligand binding to the receptor and to modify adenylate cyclase activity.[4] This finding was recently confirmed by

Magnusson *et al* using a synthetic β_1 receptor peptide as antigen in enzyme linked immunoassays.[5] It is likely, therefore, that the presence of anti-β receptor antibodies may explain the decline in the density of cardiac β receptors and the reduced inotropic responsiveness to β agonists that characterise dilated cardiomyopathy.[10] It is not known whether the presence of anti-β receptor antibodies can predict the response of cardiac β receptors to agents inducing up regulation (such as β blockers and converting enzyme inhibitors).

The pathogenesis of the antibody response in these patients remains conjectural. Immune disturbances have been described in both experimental and human cardiomyopathy and may be under genetic control. Recently, we[11] as well as others,[12] have reported that the proportion of patients with HLA-DR4 antigens is high in dilated cardiomyopathy. As it is likely that this disease is a heterogeneous entity, the patients with HLA-DR4 antigens may belong to a specific subset in which immunological abnormalities play a predominant role. This proposal is supported by our finding that most of the antibody positive patients belong to the HLA-DR4 or HLA-DR1 phenotype and that, conversely, a substantially higher proportion of HLA-DR4 patients have autoantibodies compared with those without HLA-DR4.[7] These results may be interpreted as evidence that immunogenetic factors linked to the HLA-DR system control the development of anti-β receptor antibodies. Alternatively, the association of such antibodies with HLA-DR4 may be a consequence of the link between this particular phenotype and the susceptibility to dilated cardiomyopathy. If the first alternative were true, the appearance of anti-β receptor antibodies in cardiac diseases of other aetiologies would also be linked to the HLA-DR4 phenotype whereas, if the second possibility were true, the association with HLA-DR4 would hold only for the dilated cardiomyopathy.

To examine this issue we relied on our previous findings that a small proporton of patients with ischaemic heart disease also have anti-β receptor antibodies,[4] and ischaemic cardiomyopathy is associated with a significantly increased frequency of HLA-DRw6 (but not HLA-DR4) antigen.[13] It was possible, therefore, to compare the HLA-DR dependence of anti-β receptor antibodies in these two groups of patients. The results of our study show differences in both the frequency of anti-β receptor antibodies and their HLA-DR dependence between ischaemic and dilated cardiomyopathy. About 38% of the dilated cardiomyopathy patients had antibodies detectable with the ligand binding inhibition assay compared with 22% of these with ischaemic heart disease. In both groups, there was a striking negative correlation between the presence of antibodies and the HLA-DR3 phenotype, raising the possibility that HLA-DR3 may have a protective influence against the development of autoantibodies. It is likely that the antibody response is secondary to an initial myocyte damage (neither ischaemia or cardiomyopathy) and this is more prone to occur in patients without the HLA-DR3 phenotype. The higher frequency of antibodies in dilated cardiomyopathy may reflect differences in the type of autoantigen exposure.

Associations with HLA-DR have typically been described in diseases for which an autoimmune aetiology has been either shown or strongly suggested.[14] For at least some cases of dilated cardiomyopathy, a plausible role for autoimmunity has been suggested, this involves an initial cardiac damage (possibly virus induced) that exposes autoantigens, induction of abnormal expression of class II HLA antigens, and presentation of autoantigens to T lymphocytes.[2] An immunological component to the progression of coronary artery disease has also been suggested, primarily on the basis of the presence of T lymphocytes and macrophages in atherosclerotic lesions.[15,16] A substantial proportion of these cells are immunoactivated as indicated by expression of HLA-DR and interleukin 2 receptor molecules. The extent and frequency of autoimmune responses in atherosclerotic heart disease are currently unknown. It is possible that genetic influences, operating beyond the level of the recognised "risk" factors for development of coronary artery disease, may control immune mechanisms. In our earlier study, the presence of the HLA-DRw6 phenotype in patients with ischaemic heart disease correlated most closely with family history of heart disease and not with the other risk factors, such as lipid abnormalities, diabetes, smoking, or hypertension.[13]

The different HLA-DR dependencies of anti-β receptor antibodies in the two diseases (dilated and ischaemic cardiomyopathy) would appear, at first glance, to argue against a common susceptibility of these patients to develop autoimmune responses. On the other hand, because the HLA-DRw6 phenotype, which is over-represented in ischaemic cardiomyopathy,[13] was not linked to the presence of anti-β receptor antibodies, a link between HLA-DR phenotypes and anti-β receptor antibodies and the pathogenesis of the cardiomyopathy cannot be supported. Also, as there is evidence that HLA-DR4 and HLA-DR1 share common epitopes,[17] it is likely that susceptibility to autoimmunity in dilated cardiomyopathy is linked primarily to these epitopes. If this were the case, development of anti-β receptor antibodies in dilated and ischaemic cardiomyopathy would be under the same immune genetic control. Further studies are needed to clarify this issue.

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